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Two new species of *Suillus* associated with larches in China

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Abstract —Two *Suillus* species from China, *S. alpinus* and *S. aurihymenius*, are described as new. Both *S. alpinus*, a subalpine species currently known only from southwestern China, and *S. aurihymenius*, a boreal species from northeast China, are strictly associated with larches and morphologically similar to the European *S. tridentinus* by having a viscid squamulose pileus and veiled stipe. *Suillus alpinus* is distinguished by a much duller basidiome, paler orange pores, a faint bluish discoloration of the context, and a heavily reticulate stipe apex, while *S. aurihymenius* has a reddish gold hymenium, a context that discolors a deep reddish brown, and a less squamulose pileus. The identities of the two new species and their affinity with *S. tridentinus* are supported by ITS-rDNA sequence analyses. *Suillus tridentinus*, *S. alpinus,* and *S. aurihymenius* form a monophyletic clade representing a morphologically similar European-Asian temperate lineage associated with *Larix*.

Key words — *Basidiomycota*, ectomycorrhizal fungi, phylogeny, *Suillaceae*, taxonomy

Introduction

Suillus Gray (*Suillaceae*, *Boletales*, *Basidiomycota*) comprises ectomycorrhizal fungi (EcM) associated almost exclusively with *Pinaceae* and with a host-dependent distribution (Kretzer et al. 1996; Singer 1945; Smith & Thiers 1964, 1971; Thiers 1975). The genus is ideal for studying EcM fungal phylogeny and biogeography as well as coevolution between EcM fungi and host plants (Kretzer et al. 1996, Manian et al. 2001, Mueller et al. 2001, Wu et al. 2000). Except for

a few reports, it is known that *Suillus* species mostly grow with *Pinus*, *Larix*, and *Pseudotsuga* (Alan et al. 2000; Kretzer et al. 1996; Philips 2005; Smith & Thiers 1964, 1971). Engel et al. (1996), who analyzed *Suillus* species worldwide, found that only about ten (around 5% of the described species) were symbiotic with larches. *Suillus* species associated with larches are key to understanding the diversity of the whole genus and for investigating potential coevolution between *Suillus* and theirs hosts (Kretzer et al. 1996). *Suillus* species associated with *Larix* form basal clades in the genus, suggesting that *Larix* represents an ancestral host for *Suillus* (Kretzer et al. 1996, Wu et al. 2000).

China has rather high *Larix* diversity, ranging in distribution from boreal forests in the north to subalpine regions in the south. Among 15 species of *Larix* in the world, 11 species (4 endemic, 2 introduced) are known from China (Fu et al. 1999). Some *Suillus* species associated with *Larix* have been investigated taxonomically and/or biogeographically in China. Wu et al. (2000) and Mueller et al. (2001), who explored phylogeographic relationships between North American and East Asian *Suillus* species, confirmed *S. asiaticus* and *S. cavipes* in north China. Ding & Wen (2003) enumerated 36 *Suillus* species from China, of which eight were associated with *Larix*. Our survey, which confirmed some of those species in China's larch forests, also revealed two new species that help explain *Suillus* species diversity in China and provide additional taxa for understanding the phylogeny of *Suillus* as a whole.

Materials & methods

Macro- and micro-morphological descriptions were based on fresh and dried materials respectively. Color codes follow Kornerup & Wanscher (1961). For micromorpological observations, materials were revived in 5% KOH solution for several seconds and then dyed with Congo red (aqueous reagent). Pileipellis preparations followed Wang & Verbeken (2006). Micro-morphological features were examined under a Nikon E400 microscope (1000×) and drawings were made using an installed Y-IDT drawing tube. Basidiospore observation and documentation follow Yang (2000). All specimens examined were deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS).

Total DNA was extracted from silica-dried material using a modified CTAB procedure (Doyle & Doyle 1987). The polymerase chain reaction (PCR) was performed using primer pairs ITS5/ITS4 to amplify the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (White et al. 1990). Both strands were sequenced on an ABI 3730 DNA analyzer with an ABI BigDye 3.1 terminator cycle sequencing kit (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd, Shanghai). Sequences JN201972–201976 have been submitted to GenBank. We retrieved ITS sequences of all *Suillus* species associated with *Larix* from GenBank and selected 15 most representative sequences for further phylogenetic analysis (Fig. 4). DNA sequences were aligned with

BioEdit and Clustal X with manual adjustments. *Rhizopogon subcaerulescens* A.H. Sm. and *Truncocolumella citrina* Zeller, representing the genera closest to *Suillus* (Bruns & Palmer 1989), were selected as outgroup. Phylogenetic relationships were analyzed with Maximum Parsimony (MP) using PAUP v4.0 (Swofford 2003) and with Bayesian analyses using MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003). Bayesian analyses were set with default priors, run with 4 chains for 2,000,000 generations and sampled every 100 generations.

Taxonomy

Suillus alpinus X.F. Shi & P.G. Liu, **sp. nov.** Fig. 1, 2a

MycoBank MB 816992

Differs from *Suillus tridentinus* by its association with *Larix potaninii* in southwest China, and by its duller basidiomata, less orange colored pores, faint bluish context discoloration, and heavily reticulate stipe apex.

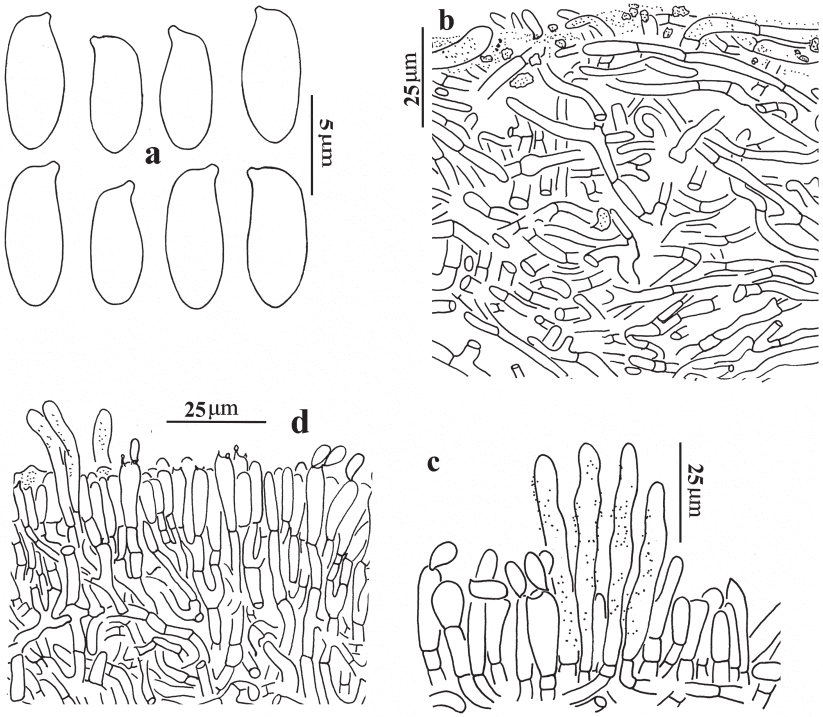

Fig 1. *Suillus alpinus* (holotype).
a. basidiospores. b. pileipellis. c. pleurocystidia. d. hymenium.

Type: China, Yunnan Prov., Shangri-La, 27°49′N 99°43′E, alt. 3600 m, scattered to gregarious under *Larix potaninii*, 16 September 2010, Shi697 (**Holotype**, HKAS 63128; GenBank, JN201974).

Etymology: *alpinus*, referring to its occurrence in subalpine regions.

Basidiomata medium-sized. Pileus 4–6.5 cm diam., convex at maturity; surface viscid, yellowish brown to pale reddish gray-brown (5D5-6E4), with grayish fibrillose squamules beneath the glutinous layer, with appendicules white to brown along margin. Stipe 40–70 × 8–15 mm, subcylindrical, equal or clavate, solid; bright yellow at apex, downwards gray brown to more reddish (6C4-6D3), white at the base; annulus well developed and persistent, superior; reticulate above the annulus; lacking glandular dots. Context 6–11 mm thick halfway to the margin in pileus, dull white, slowly turning blue or faint blue in the stipe and near the tubes when cut. Tubes 4–7 mm deep, pale dingy yellow (lighter than 4B3), becoming dingy yellowish olive (lighter than 3B3) when bruised, decurrent to stipe. Pores small, 1 per mm^2, pale dingy yellow, or concolorous with the tubes, slowly turning dingy blue when injured or in age. Odor indistinctive. Taste mild.

Basidiospores (80/3/4)10–12(–13.5) × 4–5 μm, Q = 2.20–2.80, Q_m = 2.57 ± 0.1, elongate ellipsoid, yellowish to olive-brown in 5% KOH solution, thin-walled. Basidia 22–37 × 7–9 μm, clavate, with 2–4 sterigmata. Pleurocystidia scattered, 55–73 × 8–12 μm, narrowly clavate or cylindrical; content hyaline in 5% KOH, with brownish incrustations. Cheilocystidia numerous, similar to pleurocystidia. Hymenophoral trama composed of gelatinous hyphae, slightly divergent; hyphae 4–8 μm in diameter, thin walled. Pileipellis an ixotrichoderm; hyphae 4–10 μm in diameter, thin-walled, yellowish in 5% KOH near surface with scattered brownish incrustations.

Ecology & distribution—scattered to gregarious under *Larix potaninii* in subalpine (3300–4500 m) regions of southwestern China, fruiting from May to September. Since no other tree species except *Abies* sp. was observed around the collection areas, *L. potaninii* is regarded as the host, as *Abies* is not normally regarded as a host genus for *Suillus*.

Additional specimens examined — **China**, **Sichuan**: **Derong**, Kunmu Mountain, alt. 4200 m, 20 July 2004, Z.L. Yang 4170 (HKAS 45556); **Yunnan**: **Shangri-La**, Benzilan, Baima Snow Mountains, 28°14′N 99°17′E, alt. 4200 m, 6 September 2009, X.F. Shi261 (HKAS 63133; GenBank, JN201976); Bita Lake, 27°48′N 99°54′E, alt. 3700 m, 12 August 2008, F.Q. Yu 08812004 (HKAS 63342); near Shangri-La Town, 27°49′N 99°42′E, alt. 3300 m, 21 May 2008, F.Q. Yu 08521001 (HKAS 63341).

Comments—*Suillus alpinus* is characterized by the viscid pileus with fibrillose squamules beneath the glutinous layer and veil remnants along the margin, persistent annulus, and bluish context discoloration.

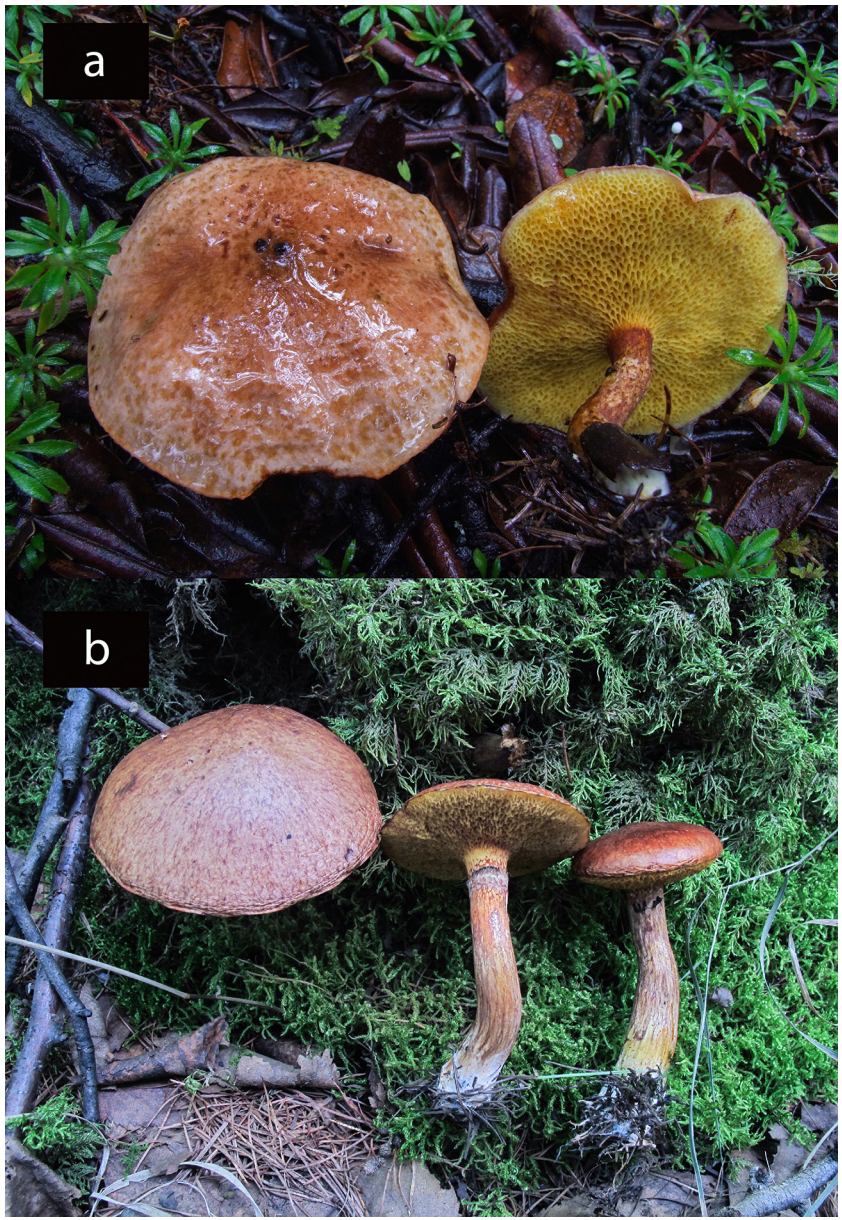

Fig 2. Habit. a. ***Suillus alpinus*** (holotype). b. ***Suillus aurihymenius*** (holotype).

Suillus aurihymenius X.F. Shi & P.G. Liu, **sp. nov**. Fig. 2b, 3

MycoBank MB 817116

Differs from *Suillus tridentinus* by its association with *Larix gmelinii* in northeast China, and by its reddish gold hymenium color, stronger reddish brown discoloration of the context, and less squamulose pileus.

Type: China. Heilongjiang: Greater Khingan Mountains Area, Huzhong Nature Reserve, 51°37′N 123°03′E, alt. 800 m, scattered to gregarious under *Larix gmelinii* (Rupr.) Kuzen., 21 August 2010, Shi597 (**Holotype**, HKAS 63129; GenBank, JN201972);

Etymology: *aurihymenius*, referring to the golden pores.

Basidiomata medium-sized. Pileus 4.4–8 cm in diameter, convex to flat at maturity; surface very glutinous when wet, ochre-yellow to cinnamon-brown or crimson-brown (darker than 7E8) with yellow brown (5E7) fibrils under the glutinous layer; appendicules whitish brown (5D5) along margin; Stipe 40–78 × 7–16 mm, subcylindrical, equal or clavate, solid; bright yellow at apex, reddish brown below or concolorous with the pileus; the membranous whitish-pink veil often leaving an apical annulus when young, nonpersistent; lacking glandular dots; pink mycelium in stipe base. Context 5–6 mm thick, yellowish white, slowly turning to light yellow (4A4) or pale orange (5A3) when cut or bruised. Tubes 5–9 mm deep, orange yellow or light yellow (4A7-4A5), becoming reddish yellow to light orange (5A5) when bruised, decurrent to the stipe. Pores small, 1 per mm^2, angular, compound, concolorous with the tubes. Odor indistinct. Taste mild; edible, as found in the local market.

Basidiospores (80/2/4) (9.5–)10–11.5 (–12) × 4–5 μm, Q = 1.96–2.31, Q_m =2.15 ± 0.18, elongate ellipsoidal, reddish brown or gray brown in 5% KOH solution, thin-walled. Basidia 25–35 × 79.5 μm, clavate, with 2-4 sterigmata. Pleurocystidia abundant, 60–76 × 9–11 μm, narrowly clavate, content hyaline in 5% KOH solution and lacking brownish incrustations. Cheilocystidia similar to pleurocystidia. Hymenophoral Trama gelatinous and divergent; hyphae 4–7 μm in diameter, thin-walled. Pileipellis a very thick layer of interwoven gelatinous hyphae 4–12 μm in diameter, thin-walled.

Ecology & Distribution — scattered to gregarious under Dahurian larch (*Larix gmelinii*) on Greater Khingan Mountain in northeastern China, fruiting during the summer and fall. As this species grows in a monocultural forest, *L. gmelinii* is proposed as the host.

Additional specimens examined — **China**, **Inner Mongolia**: **Genhe market**, 25 August 2010, Shi616 (HKAS 63130); **Argun Area**, Mordaga Native Forest Park, 51°19′N 120°40′E, alt. 540 m, scattered to gregarious under *Larix gmelinii* (Rupr.) Kuzen., 26 August 2010, Shi621 (HKAS 63131).

Comments — *S. aurihymenius* and *S. alpinus* look similar in the field, but the pileus of *S. aurihymenius* is more reddish-brown, viscid, and fibrillose or

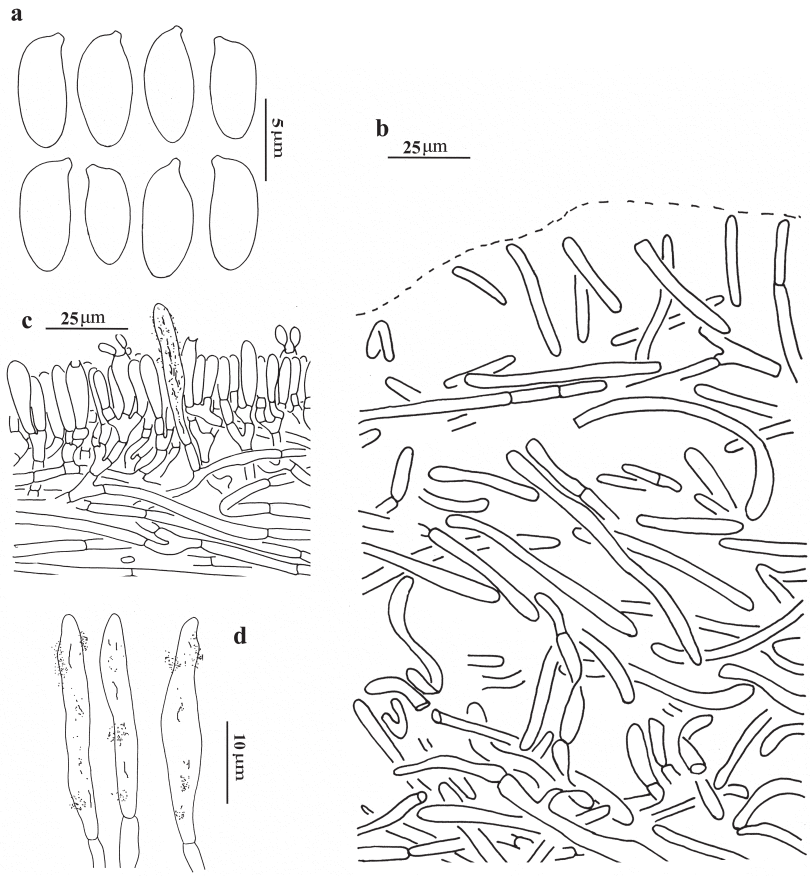

Fig 3. *Suillus aurihymenius* (holotype).
a. basidiospores. b. pileipellis. c. hymenium. d. pleurocystidia.

squamulose than that of *S. alpinus,* and the context of *S. aurihymenius* does not change to blue when bruised.

Phylogenetic results

ITS-rDNA sequences were obtained for *S. alpinus* and *S. aurihymenius.* The molecular dataset consisted of 22 OTUs and 731 characters, of which 31 were ambiguous and excluded from the analyses. Of the remaining 653 characters, 529 characters were constant, 92 were uninformative, and 110 were parsimony-informative. Parsimony analysis produced one most parsimonious tree of 1000

replications with consistency index (CI) = 0.743, retention index (RI) = 0.796, and rescaled consistency index (CI) = 0.257. The tree is shown with bootstrap values (>60%) in Fig. 4. Topology of the Bayesian tree is the same as the MP tree, and posterior probability values are included at each node in Fig. 4. *Suillus alpinus* forms a monophyletic clade with 100% bootstrap and 1.0 posterior probability supports. The *S. aurihymenius* monophyletic clade was supported with 99% bootstrap value and 0.96 posterior probability. Also the two new species and *S. tridentinus* forms a monophyletic clade with 92% bootstrap support and 1.0 posterior probability.

Discussion

Suillus alpinus and *S. aurihymenius* appear similar in the field and are both characterized by a viscid pileus with fibrillose squamules beneath the glutinous layer, veil remnants hanging from the margin, and a veiled stipe lacking any glandular dots. They match the morphological and ecological descriptions of *Suillus* sect. *Larigni* (Singer 1986). Both species also morphologically close to the European *S. tridentinus* (Bres.) Singer. Compared with other *Suillus* species, all three species have very long spores, often exceeding 10 µm (Moser 1983). Kretzer et al. (1996), who investigated the phylogenetic diversity of *Suillus* species associated with *Larix*, identified two well-supported clades excluding *S. spectabilis*. Our analyses strongly support the Asian *S. alpinus* and *S. aurihymenius* in a monophyletic clade with the European *S. tridentinus*. Species in this monophyletic clade can be separated morphologically from *S. grevillei* and other species in its sister clade containing by the presence of squamules beneath the glutinous layer, veil remnants on the pileal margin, and much darker basidiomata.

It is interesting to note that *S. aurihymenius*, which grows in northwestern China, shows a closer affinity to the European *S. tridentinus* than to the southwestern Chinese *S. alpinus* in the phylogenetic tree. This relationship is similar to the situation in *Chroogomphus* (Li et al. 2009), where northern Chinese samples are conspecific with European samples while *Chroogomphus* samples from southwestern China represent a separate sibling species. Phylogenetic and morphological comparisons suggest that *S. aurihymenius* and *S. tridentinus* are sister species. The high genetic similarity between European and northern Chinese taxa might have been facilitated by the dispersal between different populations and thus contributed to the low genetic divergence (Petersen & Hughes 2007). As has been shown in other fungal groups (Ge et al. 2008, Li et al. 2009, Zhang et al. 2004), comparison analyses of counterparts or conspecific species in China's northern and subalpine regions would help elucidate EcM fungal diversification and fill geographic sampling gaps in temperate regions.

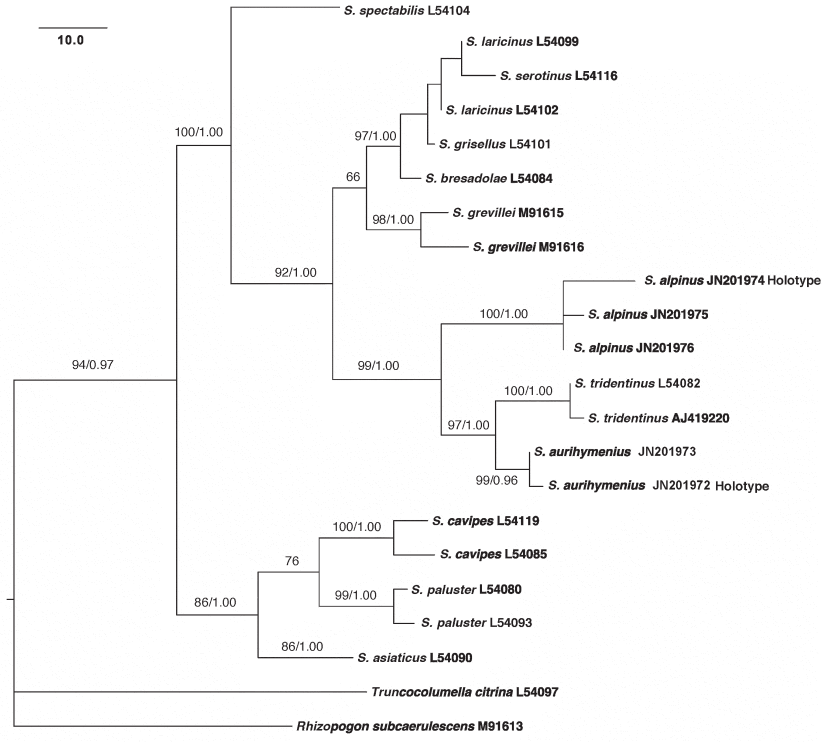

Fig 4. Phylogenetic tree resulting from Maximum Parsimony analysis of partial nuclear subunit ribosomal DNA sequences (ITS) of *Larix*-associated species of *Suillus*. Numbers above branches are bootstrap values >50%. Values after the slash are posterior probability values obtained through Bayesian analysis of the same data set. The sequences *S. alpinus* and *S. aurihymenius* were generated in this study; all other sequences were obtained from Kretzer et al. (1996) except for *S. grevillei* (Baura et al. 1992) and AJ419920 and M91613 (Martín & Raidl 2002).

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